

THE PHAGE COLLECTION PROJECT

PHAGE ISOLATION COURSE

STUDENT HANDBOOK

STUDENT NAME:

TUTOR GROUP:



WELCOME

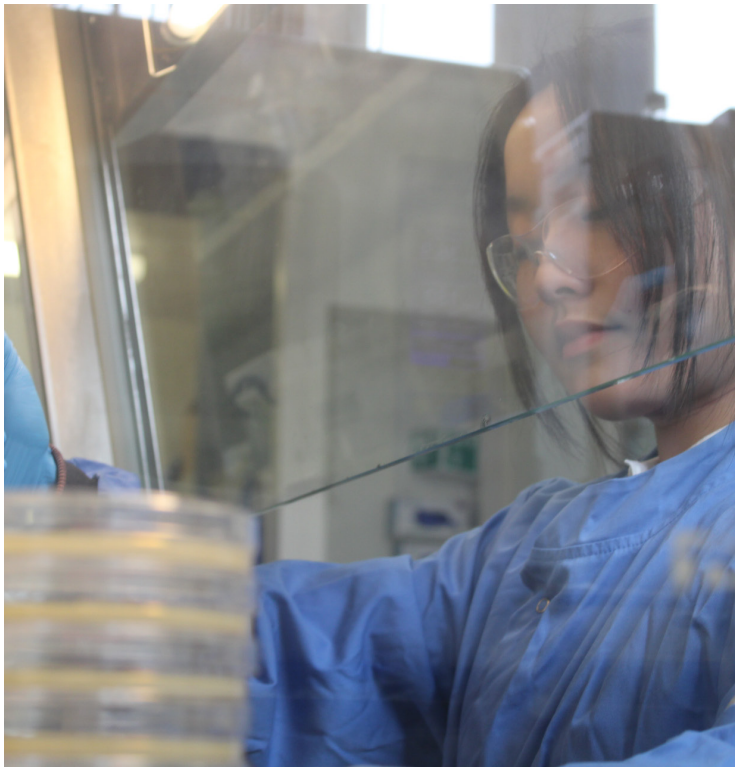
Welcome to The Phage Collection Project's Phage Isolation Course, an 11-week programme designed to enable you to isolate your very own phage in your school laboratory.

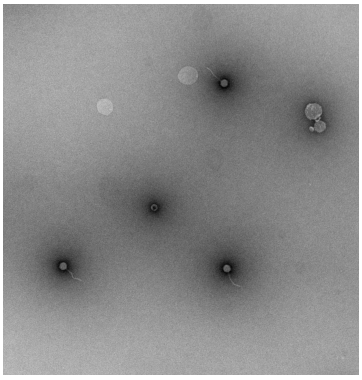
The Phage Collection Project is a citizen science initiative based at the University of Southampton. Our project aims to empower citizen scientists and academics to collectively advance phage research and therapeutics to help combat antibiotic-resistant infections.

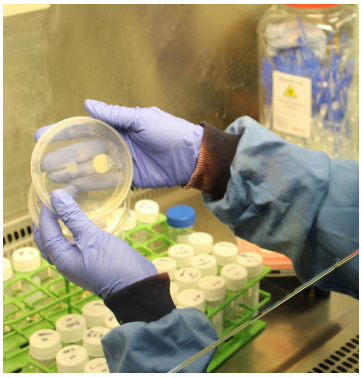
Bacteriophages (phages for short) are viruses which only infect and kill bacteria. They are the most abundant entity on Earth, found in any environment where bacteria reside such as lakes, soil or even the human gut.

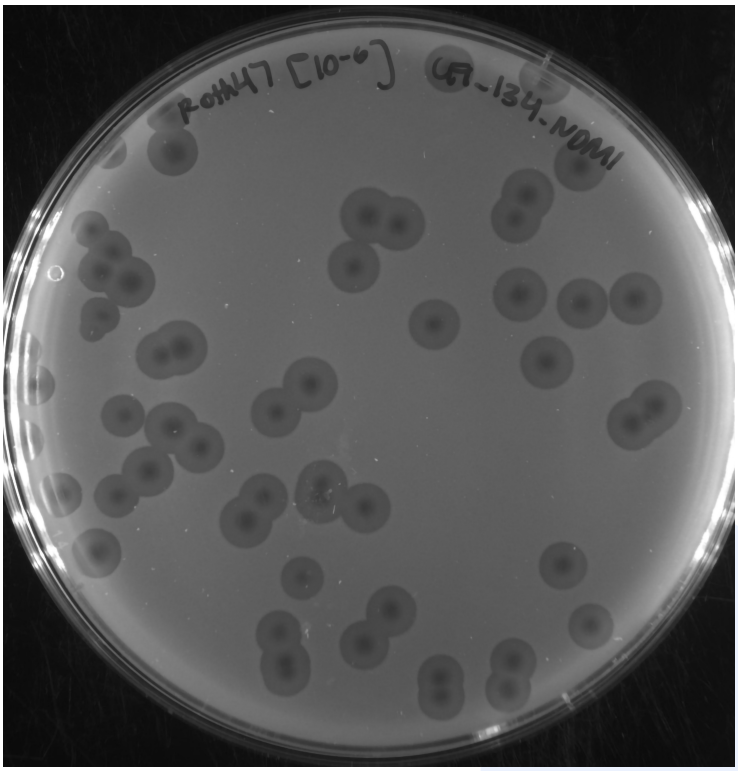
Over the next 11 weeks you are going to take part in active scientific research. At the end of this course, we hope that you will not only learn more about phages and gain new laboratory skills, but also be able to isolate and name your very own phage which will be added to The Phage Collection Project Biobank.

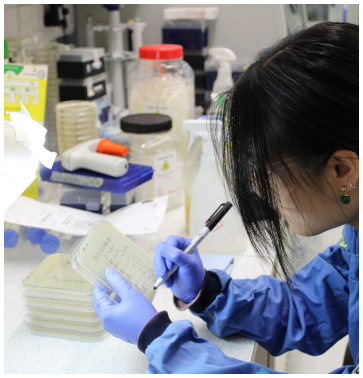
Our goal is to utilise phages in our biobank to advance phage research and formulate therapies which can be used to treat patients struggling with antibiotic-resistant infections.













THE PHAGE COLLECTION PROJECT

BACKGROUND

Phages are viruses which only infect and kill bacteria. Unlike other viruses such as SARS-CoV-2, phages do not infect or damage humans cells, enabling us to utilise them in therapeutics to treat bacterial infections.

These tiny entities are the most abundant biological entity found on this planet, estimated to outnumber bacteria 10 to 1[1]. We can find phages in environmental sources, our own bodies and on surfaces. In this course, you will be isolating phages from 50 mL water samples collected from your local environment.

To view the phages that we isolate, we need to use a transmission electron microscope (TEM) which uses electrons, rather than light to view the specimen.

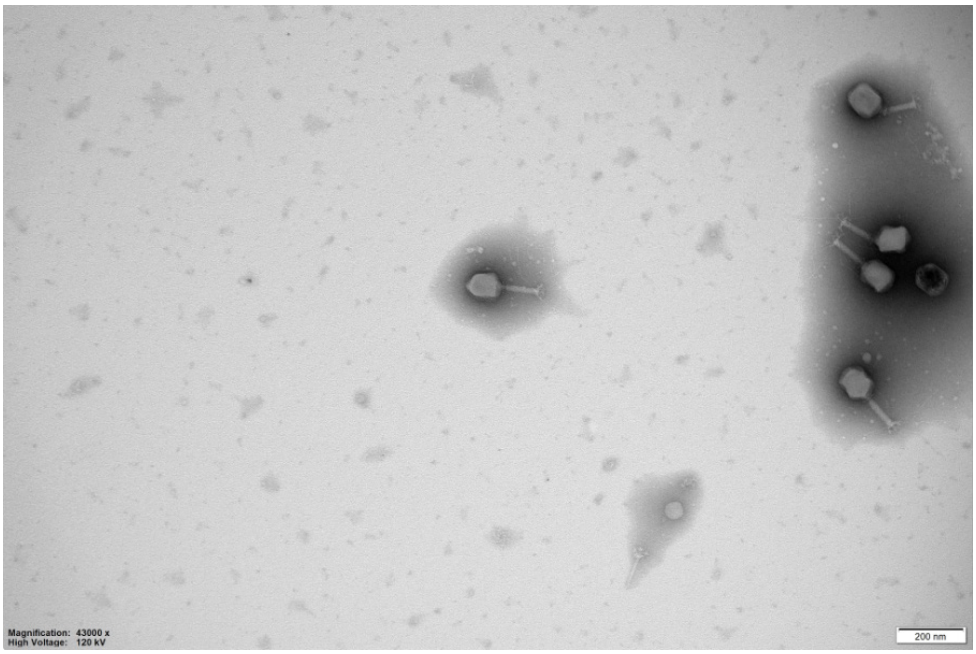


Figure 1: A transmission electron microscopy image of phage ROTH78 which infects and kills the bacteria *Klebsiella pneumoniae*. *K. pneumoniae* is a pathogen which can cause infections like urinary tract infections (UTI). Increasingly, this bacterial species displays resistance against different antibiotics, consequently causing chronic infections for patients. Phages like ROTH78 may provide an alternative strategy for effectively treating strains of *K. pneumoniae* resistant to antibiotics.

Caudoviricetes is the class of phage that we typically isolate in the highest abundance[2]. Within this class there are various phage morphologies that we observe including myophage, siphophage and podophage, which are all double stranded DNA phages[3].

Each of these phages share structural components essential for their survival and replication:

Capsid: Contains and protects the phage's genetic material.

Collar: Connects the capsid and sheath together and controls the release of the phage's genome from the capsid upon injection.

Tail fibres: Interact with the bacterial cell membrane to determine if it is a suitable host for the phage.

Base plate: Meets the bacteria's cell membrane and enables the phage to inject its genetic material into the host cell.

Tail spikes: Recognise and bind to receptors on the bacteria's cell membrane.

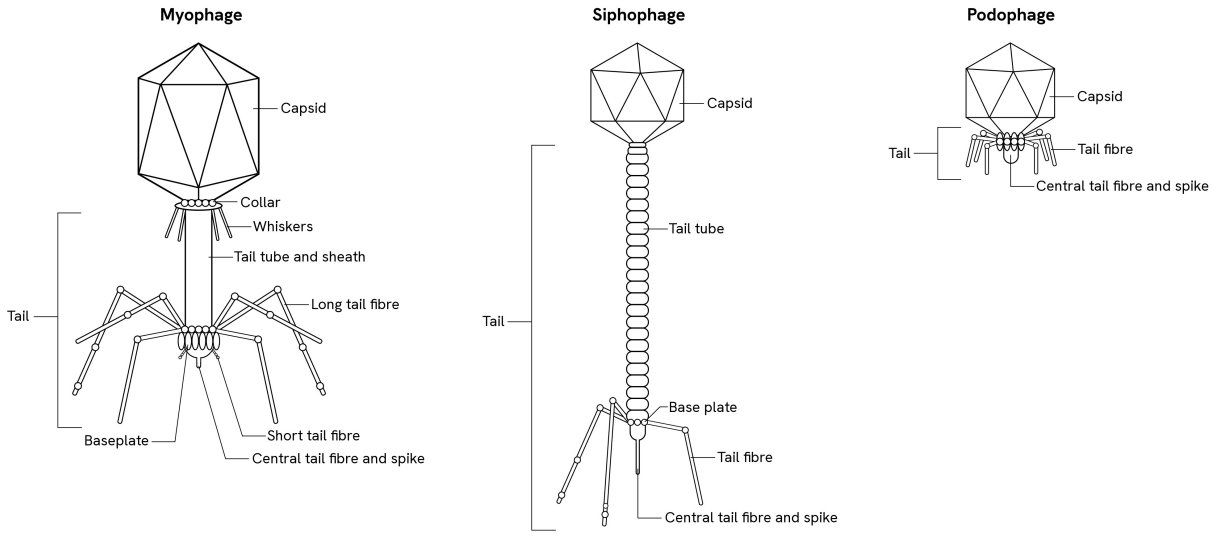


Figure 2: Morphologies of the myophage, siphophage and podophage. Figure taken and adapted from Nobrega *et al* 2018[3]

Phages can undergo four lifecycles: lytic, lysogenic, pseudolysogenic and chronic. Lytic phages undergo the lytic lifecycle. Temperate phages follow the lytic, lysogenic and pseudolysogenic lifecycles. Filamentous phages follow the chronic lifecycle. When we design phage therapies, we use lytic phages which kill the bacteria.

Lytic lifecycle

1. A phage encounters, recognises and binds to a bacteria **reversibly** using its tail fibres.
2. Using its tail spikes, the phage binds **irreversibly** to the bacteria's cell surface.
3. Once bound securely, the phage injects its genetic material into the bacterial cell.
4. The phage hijacks and takes control of the cell, replicating and manufacturing each of its structural components within the cell.
5. Once all the structural components are created, they assemble to form a fully functional phage.
6. The phages produce enzymes which degrade the bacterial cell wall and membrane, causing the bacteria to die and releasing the phages into the surrounding environment.

Lysogenic lifecycle

7. A phage encounters, recognises and binds to a bacteria **reversibly** using its tail fibres.
8. Using its tail spikes, the phage binds **irreversibly** to the bacteria's cell surface.
9. Once bound securely, the phage injects its genetic material into the bacterial cell.
10. The phage's genetic material becomes integrated into the bacteria's genome, forming a **prophage**.
11. The bacteria continues to live and multiply. When the bacteria multiples the prophage is replicated and passed to the daughter cells.
12. Under certain conditions the prophage becomes active and removes itself from the bacterial genome.
13. The lytic cycle is initiated.

Pseudo-lysogenic lifecycle

14. A phage encounters, recognises and binds to a bacteria **reversibly** using its tail fibres.
15. Using its tail spikes, the phage binds **irreversibly** to the bacteria's cell surface.
16. Once bound securely, the phage injects its genetic material into the bacterial cell.
17. The phage's genetic material sits within the bacteria's cytoplasm as an extrachromosomal element.
18. The bacteria continues to live and multiply. When the bacteria multiples the phage genome is passed asymmetrically to one of the daughter cells.
19. Under certain conditions the phage will leave the pseudo-lysogenic lifecycle and follow either the lytic or lysogenic lifecycle.

Chronic lifecycle

1. A phage encounters, recognises and binds to a bacteria **reversibly** using its tail fibres.
2. Using its tail spikes, the phage binds **irreversibly** to the bacteria's cell surface.
3. Once bound securely, the phage injects its genetic material into the bacterial cell.
4. The phage hijacks and takes control of the cell, replicating and manufacturing each of its structural components within the cell.
5. Once all the structural components are created, they assemble to form a fully functional phage.
6. The phage exits the bacteria through a secretion channel in the bacteria's cell membrane, preventing cell lysis.
7. Occasionally the phage will enter spontaneous lysis, causing bacterial cell death which promotes biofilm formation.

Biofilms are complex microbial communities that are responsible for causing chronic infections, particularly lung infections for cystic fibrosis patients. The matrix that surrounds the microorganisms in a biofilm can reduce antibiotic penetration, making these microbial communities very difficult to treat and eliminate. Phages in the chronic lifecycle, which stimulate spontaneous lysis, promote biofilm formation by increasing the structural integrity of the matrix[4].

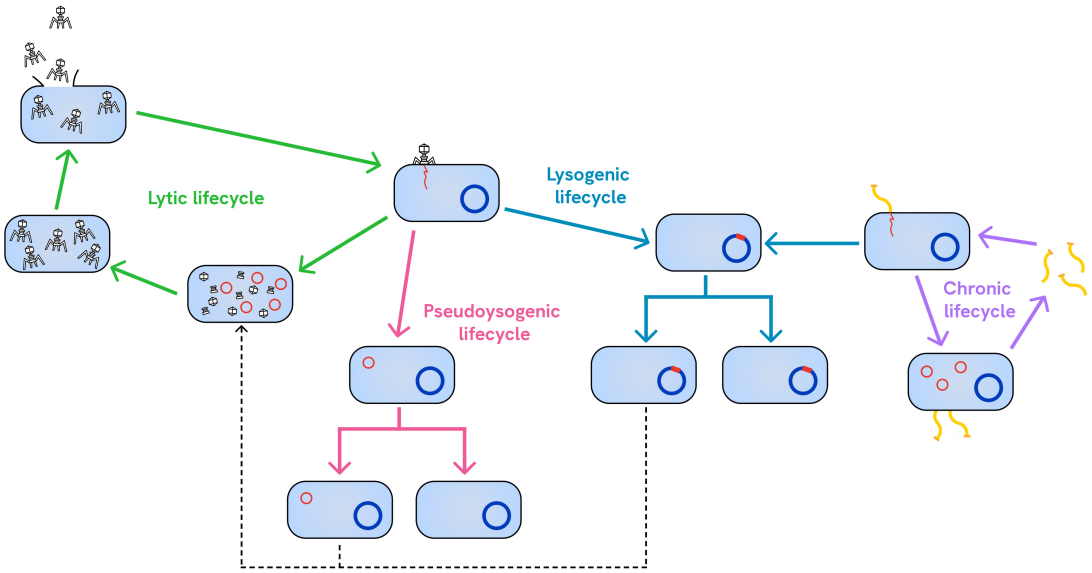


Figure 3: The four phage lifecycles: Lytic, lysogenic, pseudolysogenic and chronic.

The action of phages is first thought to have been observed in 1895 by bacteriologist Ernst Hankin when analysing the waters of the Ganges and Jumuna rivers[5]. Two decades later, phages were described independently, first in 1915 by William Twort and then in 1917 by Felix d'Herelle[6].

Phage therapies use phages to treat bacterial infections. This medicine has been used for over a century, first applied to treat patients suffering from dysentery in 1919[7]. In 1928, when antibiotics were discovered, these antimicrobial drugs became more popular than phage therapy in the Western world. However, in Eastern Europe they continued to apply phage therapy. Because of the global health crisis posed by antimicrobial resistance, interest in phage therapy is now returning.

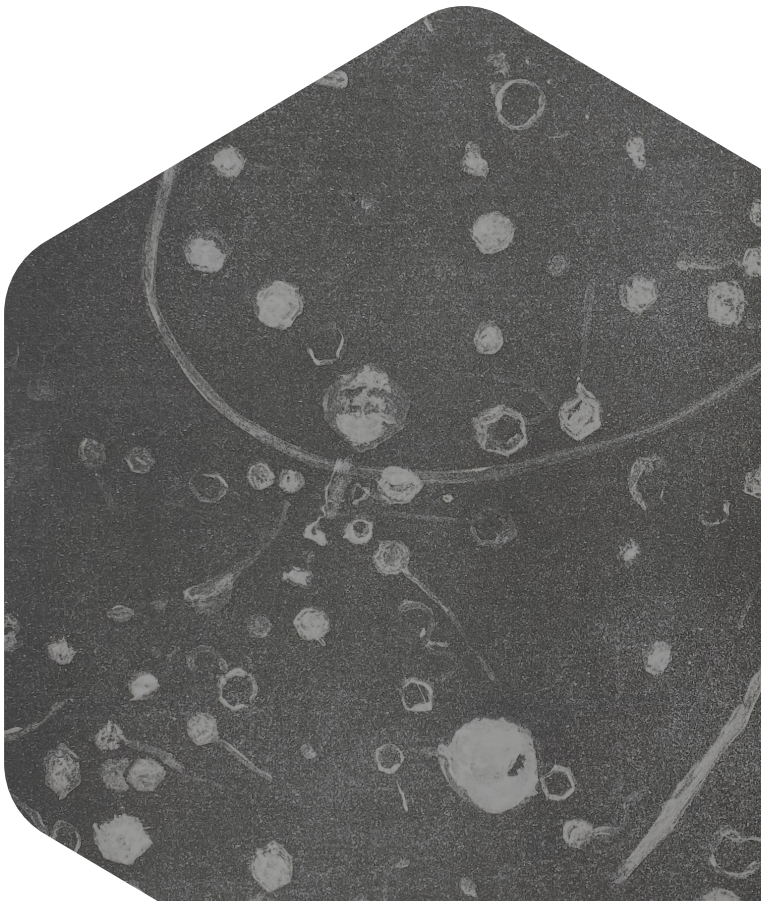
When we become unwell due to a bacterial infection, antibiotics are often prescribed to treat the condition. However, misusing and overusing antibiotics can cause bacteria to develop resistance to them, meaning that the antibiotics are no longer effective at eliminating and treating an infection. Antibiotic resistance poses a major problem globally. If bacteria become resistant to antibiotics, we may face a situation like the time before antibiotics were discovered – we may not be able to fight off infections, and serious medical treatments like organ transplants and chemotherapy may become more dangerous to complete.

This highlights the importance of researching and developing new antibacterials such as phage therapy to enable us to successfully treat bacterial infections in the future.

For further information on phages and microbiology please look at the textbook 'Brock biology of microorganisms'[8] or check out The Phage Collection Project website.

MATERIALS

- 50 mL falcon tubes
- Gloves (XS, S, M, L)
- Hand sanitising wipes
- 70% ethanol
- Blue roll
- Bunsen burner
- Heatproof mat
- Matches
- 10 mL serological pipette
- 10 mL serological pipette tips
- 2 mL tube
- 2 mL tube rack
- Pen
- Centrifuge (microcentrifuge/tabletop centrifuge)
- 0.22 µM PES filter
- 5 mL syringe
- 50 mL falcon tube rack
- 20 mL syringe
- 500 mL flask
- LB broth
- Commensal Category 1 *Escherichia coli* K-12 strain (Reference no. LZB 035)
- P200 pipette
- P200 pipette tips
- Incubator (25℃) (Rocking incubator optional)
- Fridge (4℃)
- Autoclave
- Water bath (55℃) or microwave for top agar
- 0.7% LB top agar
- LB agar plates (LBA)
- P10 pipette
- P10 pipette tips
- Parafilm
- λ phage stock
- 96-well plate
- Ruler
- Sterile toothpicks
- Sterile tweezers
- Sterile paper strips
- Scientific calculator



Artwork by Catherine McColgan. Etching capturing what phages look like under a transmission electron microscope.

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GLOSSARY

Agar plate: A sterile petri dish that contains a solid growth medium referred to as agar which is used to culture microorganisms

Antibiotic resistance: Bacteria evolving mechanisms to overcome and survive antibiotic treatment

Aseptic conditions: A state where there are few or no microorganisms in an environment to minimise the risk of contamination

Autoclave: A machine that uses steam under pressure to clean and sterilise equipment

Bacterial lawn: A layer of bacteria that covers the surface of an agar plate

Centrifugation: A process that separates the components of a mixture based upon their density

Colorimeter: A piece of equipment that measures the amount of light that passes through a certain sample

Culture: A collection of organisms grown on a nutrient rich medium[8]

Death phase: The final stage of the microbial growth cycle, where cell death causes a decline in the population[8]

***Escherichia coli* K-12:** A gram-negative, non-pathogenic laboratory bacterial strain

Exponential phase: The growth phase during the microbial growth cycle, where the microorganism population replicates and doubles at regular time periods[8]

Flicking: Interaction in tube for gentle mixing of components

Host cell: A cell which viruses use to replicate

Incubate: Maintaining a controlled environment for microorganisms such as bacteria to grow

Inoculate: When you introduce microbes to a culture media such as agar where they will reproduce

Lag phase: The period between the inoculation of a microorganism and the initiation of growth during the microbial growth cycle[8]

LB media: A nutrient rich medium which is used to culture bacteria

Optical density: A measurement of how much light scatters through a sample of microorganisms in a solution

PES filter: Used to separate fine particles using a material which has a low affinity to proteins

Phage: A virus which infects and replicates in bacterial cells

Phage plaque: A clear region on a bacterial lawn which indicates the presence of phages

Serial dilution: A series of stepwise dilutions which reduce the concentration of a particular substance/molecule/microorganism within a solution

Spotting: A technique that involves placing a sample of a phage onto a bacterial lawn to see whether it infects and kills the bacteria

Stationary phase: The third phase of the microbial growth cycle, where there is no net increase of decrease in the population[8]

Supernatant: Top liquid portion of the tube after centrifugation

Virion: The infective form of the phage, outside of the host cell

96-well plate: Container with wells to store phages or phage dilutions

LEARNING OUTCOMES

By the end of the 11 week course you should be able to achieve the seven learning outcomes listed below:

1. Explain what bacteriophages (phages) are and what they infect

2. Identify four different ways phages interact with bacteria (phage lifecycles)

3. Safely collect and prepare a sample that may contain phages

4. Grow bacteria and phages together to test whether phages are present

5. Observe and describe clear areas (plaques) made by phages on bacteria

6. Count plaques to estimate how many phages are in a sample

7. Use aseptic techniques to work safely and avoid contamination

SCALE

Before starting the course, it is important to understand the measurements that we are going to be using. Throughout the course you will be measuring volumes in microlitres (µL). This is a metric unit of measurement for liquid volume. 1 µL is equivalent to 0.001 mL.

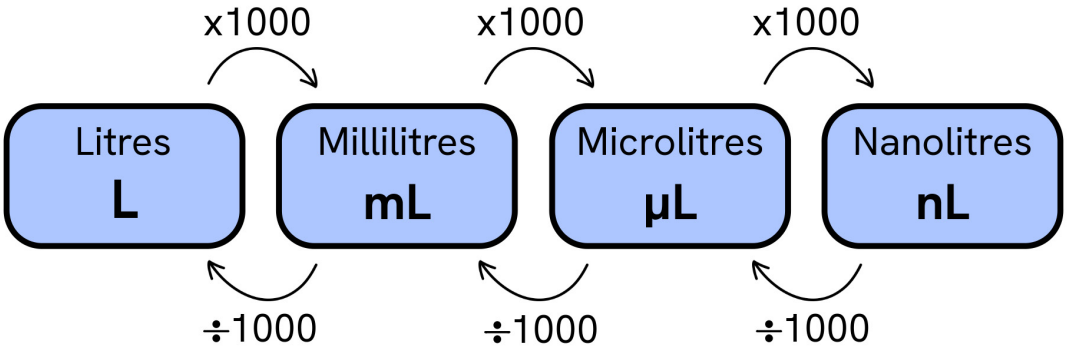


Figure 4: A conversion scale for metrics of liquid volumes.

Throughout the course you will also see the micrometre (µm) and nanometre (nm) measurement. 1 µm is equivalent to 0.001 mm. 1 nm is equivalent to 0.000001 mm.

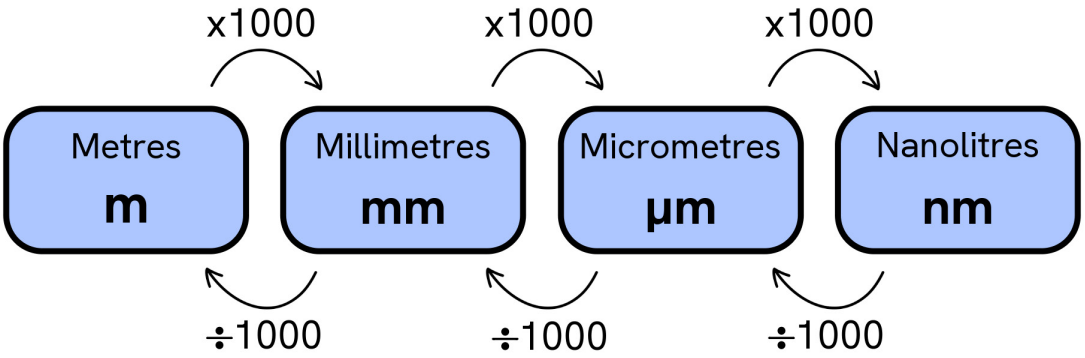
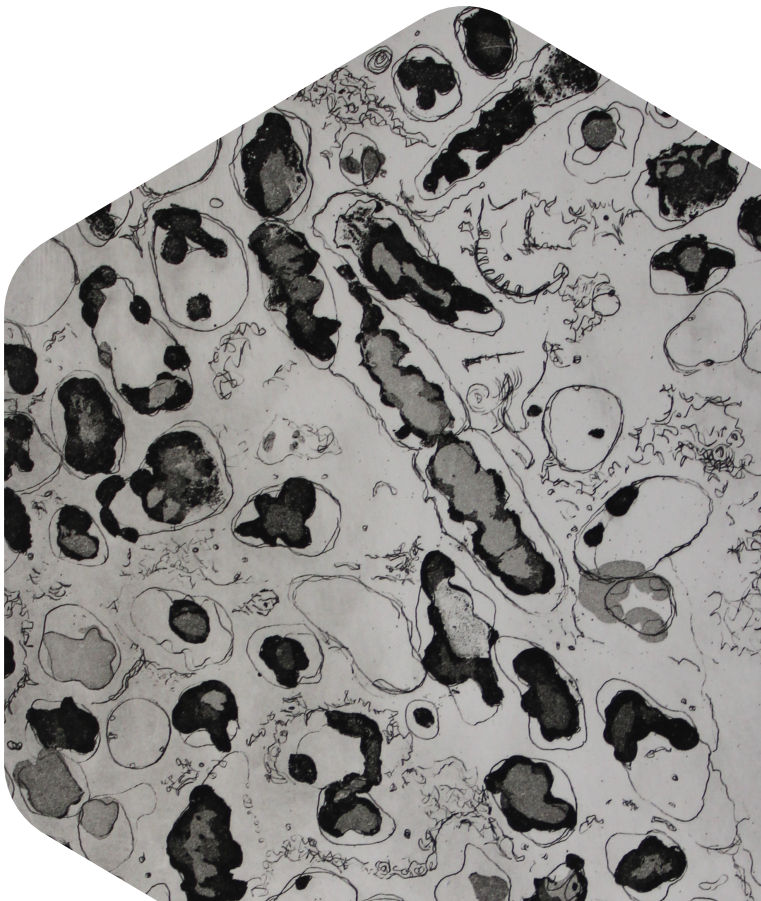


Figure 5: A conversion scale for metrics of length.



Artwork by Catherine McColgan. Etching capturing the process of plasmolysis, a technique that bacteria use to defend themselves against phage infection.

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BEFORE STARTING THE COURSE

Prior to starting the Phage Isolation Course, you need to collect a 50 mL sample of water. Your teacher or laboratory technician will provide you with a 50 mL falcon tube, a pair of gloves and a sanitising wipe to collect your water sample. Make sure you are supervised when collecting your sample at home.

Before collecting your sample of water make sure you and your parent/guardian (if under 18 years of age) have read the Phage Isolation Course Participant Information Sheet and signed the consent form. To register onto the Phage Isolation Course and access the Participant Information Sheet and consent form, ask your teacher for the link to The Phage Collection Project Portal.

Collect a 50 mL sample of water from your local environment using the falcon tube, gloves and sanitising wipe provided. After sealing the falcon tube securely, wipe down it down with the sanitising wipe. Dispose the sanitising wipe and gloves responsibly in a bin. Record the GPS coordinates of the sampling location using a smartphone and details about the sampling site (e.g. time of day, weather conditions etc).

Please ensure you follow the safety information below when collecting your water sample:

- Ensure you are wearing appropriate clothing and footwear to reduce the risk of slips, trips and falls.
- There might be environments where you are legally not allowed to collect water samples. Make sure to check local and provincial laws in the environment you intend to collect your water sample from.
- Please use the gloves provided by your teacher to collect your water sample. The gloves may contain latex. If you are allergic to latex, please discuss this with your teacher.
- Wash your hands thoroughly with soap and water after collecting your sample.
- Cover any wounds/cuts/grazes.
- Do not drink the water.
- Collect your water samples in pairs or groups.
- Do not collect any vegetation of animals. Make sure to leave the environment as you found it.

Instructions for how to find GPS coordinates on your smartphone are provided below.

Apple devices:

1. Open Apple Maps and have location settings turned on.
2. Press and hold on the sampling location on Apple Maps.
3. A grey box will appear on the bottom half of your screen.
4. Scroll up and find the coordinates.
5. Screenshot the coordinates to save for later when you fill out the sampling information sheet.

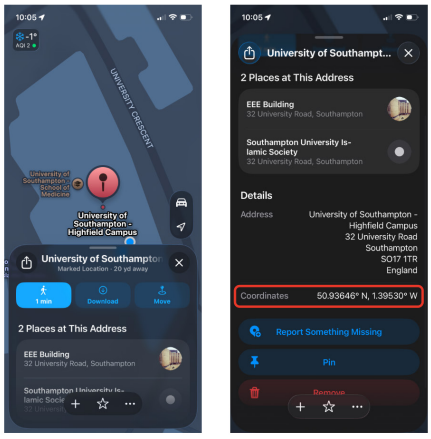


Figure 6: How to find GPS coordinates on Apple devices.

Instructions continued on the next page...

Android devices:

1. Open Google Maps with location settings turned on.
2. A blue circle will appear which shows your current location.
3. Zoom into your current location (blue circle) until you cannot zoom any further.
4. Press and hold on the blue circle.
5. A box will appear titled ‘Dropped Pin’.
6. Scroll up to find the coordinates of your sampling location.
7. Screenshot the coordinates to save for later when you fill out the sampling information sheet.

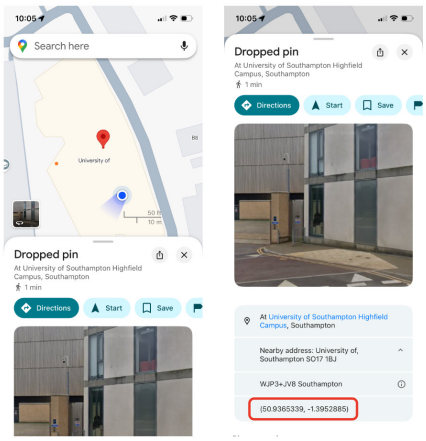


Figure 7: How to find GPS coordinates on Android devices.

Once you have collected your sample and sampling information, log it on The Phage Collection Project Portal by following the instructions below:

1. Go to The Phage Collection Project Portal by scanning the QR code below.
2. Once you have logged in to your account, log your sample by pressing the ‘Register Kit’ button. The portal will ask you to input your kits unique ID no. (e.g. SRR25-0000). Once logged, your kit will appear under your orders profile.
3. To input your kits sampling information, press the ‘Collect’ button under the Actions tab.
4. Fill out the sampling information form, providing details such as the date of sample collection and GPS coordinates.
5. Once you have completed the form press ‘Save’.
6. The status of your kit will change to ‘Collected’.

Bring your 50 mL water sample into school before you start the Phage Isolation Course.

LABORATORY SAFETY

When we conduct experiments it is essential that we take all necessary precautions to ensure that we and others remain safe in the lab.

Throughout the Phage Isolation Course make sure that you listen and follow all instructions provided by your teacher and laboratory technician.

Below we have provided some safety guidance which you should follow when completing the course:

- In the lab you must wear your protective personal equipment (PPE) – lab coat, goggles, gloves and closed toed shoes.
- Ensure hair it tied back.
- If you notice a spill, bring this to the attention of your teacher and laboratory technician.
- Cover all wounds/cuts/grazes.
- Before and after your experiments, wipe down the benchtop with 70% ethanol.
- Do not drink the 70% ethanol and avoid contact with the eyes and skin.
- Wash your hands thoroughly with soap and water before and after completing your experiments.
- Do not drink the *E. coli* K-12 culture or 50 mL water sample.

GENERAL LAB SKILLS

Over the next 11 weeks, you will be repeating some key laboratory skills, essential to ensure accurate phage isolation. Before starting the Phage Isolation Course, follow the videos below, by scanning the QR codes, to familiarise yourself with some of the key skills that you will learn and use throughout the course.

Pipetting

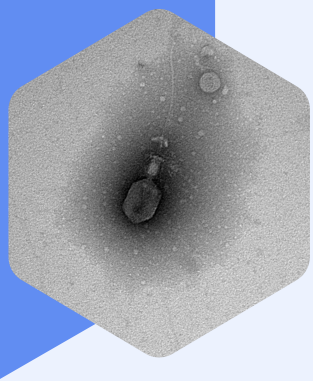
Labelling your sample

Centrifugation

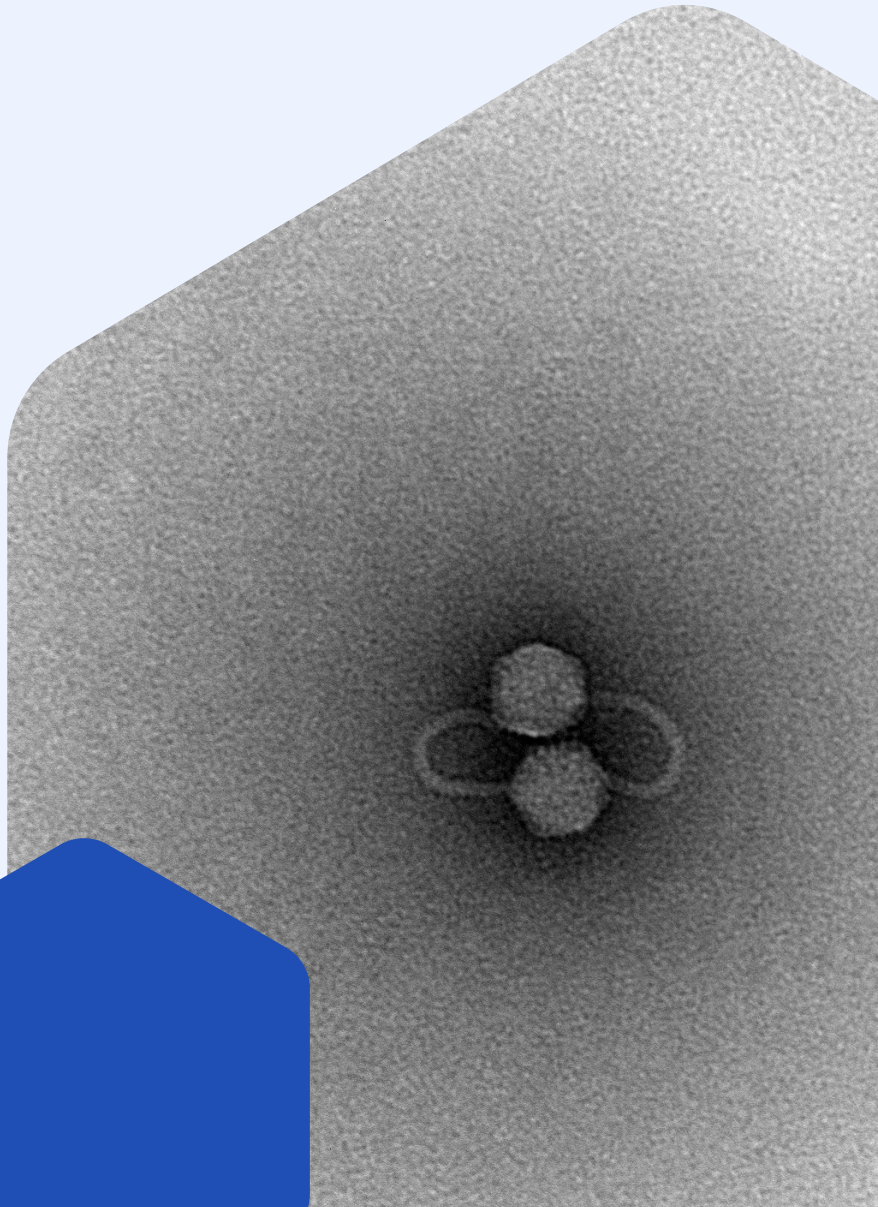
Pouring the supernatant

Aseptic techniques

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WEEK 1: ENRICHMENT

Equipment:

- 50 mL falcon tube
- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- 10 mL serological pipette
- 10 mL serological pipette tips
- Pen
- 0.22 µM PES filter
- 50 mL falcon tube rack
- LB broth
- Commensal Category 1 *Escherichia coli* K-12 strain (Reference no. LZB 035)
- P200 pipette
- P200 pipette tips
- Incubator
- Fridge

Microcentrifuge:

- 2 mL tubes
- 5 mL syringe
- 2 mL tube rack

Tabletop centrifuge:

- 20 mL syringe

This week you are going to start preparing the sample of water you collected for phage isolation. You will remove any sediment that might be in your water sample using centrifugation and filtration.

Before starting any experiment, it's important to first set up and light a Bunsen burner on your bench. The flame of the Bunsen burner creates an updraft which pushes contaminants like bacteria away from the benchtop, helping to create more sterile working conditions. (To learn more about aseptic techniques, see 'General Lab Skills').

Now that the Bunsen burner is set up, it is time to centrifuge and filter your water sample.

If you are using a tabletop centrifuge skip to step B. If you are using a microcentrifuge follow step A.

Step A: Centrifuge and filter (microcentrifuge)

21. Using the 10 mL serological pipette, pipette 2 mL of your water sample into a 2 mL tube.
22. Repeat step 1 so that you have two 2 mL tubes of your water sample (total 4 mL).
23. Seal both tubes and label with your initials, date and unique sample ID. (For guidance on how to correctly label a sample, see 'General Lab Skills').
24. Place your 2 mL tubes in the centrifuge.

    IMPORTANT: Make sure the centrifuge is balanced. (For guidance on how to balance a centrifuge, see 'General Lab Skills').

5. Centrifuge your water samples at 3000x g for 5 minutes at 4°C.
6. Open the packaging containing the 0.22 µM PES filter, but leave the filter inside the packaging.
7. Place the 5 mL syringe in the PES filter (set the plunger back inside the syringe packaging to maintain aseptic conditions).
8. Place the filter and syringe over an open, clean 50 mL falcon tube.
9. Pour the supernatant from both 2 mL tubes into the syringe.
   IMPORTANT: Only pour the supernatant into the syringe. Do not pour the pellet at the bottom of the 2 mL tubes. (For guidance on how to correctly pour the supernatant, see 'General Lab Skills').
10. Deplunge the syringe, passing the supernatant through the filter and into the falcon tube.
11. Seal the falcon tube.
12. Write your initials, the date and unique sample ID on the falcon tube containing your filtered water sample and place it in a falcon tube rack.

Scan to watch a video tutorial on Step A

Step B: Centrifuge and filter (tabletop centrifuge)

1. Place your 50 mL water sample into the centrifuge.
   IMPORTANT: Make sure the centrifuge is balanced. (For guidance on how to balance a centrifuge, see 'General Lab Skills').
2. Centrifuge your water sample at 3000x g for 5 minutes at 4°C.
3. Open the packaging containing the 0.22 µM PES filter, but leave the filter inside the packaging.
4. Place the 20 mL syringe in the PES filter (set the plunger back inside the syringe packaging to maintain aseptic conditions).
5. Place the filter and syringe over an open, clean 50 mL falcon tube.
6. Pour the supernatant from the falcon tube into the syringe.
   IMPORTANT: Only pour the supernatant into the syringe. Do not pour the pellet at the bottom of the falcon tube. (For guidance on how to correctly pour the supernatant, see 'General Lab Skills').
7. Deplunge the syringe, passing the supernatant through the filter and into the falcon tube.
8. Seal the falcon tube.
9. Write your initials, the date and unique sample ID on the falcon tube containing your filtered water sample and store it in a falcon tube rack.

Scan to watch a video tutorial on Step B

Centrifugation spins samples at high speeds. This causes denser particles in a mixture to settle at the bottom of the tubes (the pellet), while lighter particles stay at the top (supernatant). Because phages are very small they will stay in the supernatant after centrifugation.

Polyethersulfone (PES) membranes with a 0.22 µM pore size will remove finer particles, bacteria and fungi that were not fully removed by centrifugation. These particles will be stuck in the filter, while phages which are smaller than the filters pore size can pass through. This creates your filtered water sample.

Step C: Enrichment

1. Add 10 mL of LB broth into a 50 mL falcon tube using the 10 mL serological pipette.
2. Add 100 µL of the *Escherichia coli* K-12 culture to the same falcon tube with a P200 pipette.
3. Using the 10 mL serological pipette, transfer 1 mL of your filtered water sample into the falcon tube.
   IMPORTANT: Change the serological pipette tip between steps 1 and 3.
4. Write your initials, the date and unique sample ID on the falcon tube containing your enriched phage sample.
5. Incubate the falcon tube at 25°C overnight.
   OPTIONAL: Incubate with rocking if available.
6. Save your remaining filtered water sample. Store in a 4°C fridge.

Scan to watch a video tutorial on Step C

E. coli K-12 is a gram-negative bacterial strain which was isolated over 100 years ago and is a commonly used model organism in laboratories[9]. Most strains of *E. coli* are commensals, which provide mutual benefits to their hosts[10], such as *E. coli* that naturally reside in the human gastrointestinal tract. The *E. coli* K-12 strain (Reference no. LZB 035) used in this course is a commensal. Some *E. coli* strains are pathogenic and are increasingly displaying resistance to antibiotics. Between 2019 and 2024, *E. coli* was responsible for 65% of antibiotic-resistant bloodstream infections in England[11] and has been named as a critical priority pathogen by the World Health Organization[12].

If a phage that targets *E. coli* K-12 is present in your water sample the phage will replicate and you will be able to detect it later throughout the course.

NOTES

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WEEK 2: SPOT TEST

Equipment:

- 50 mL falcon tube
- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- 10 mL serological pipette
- 10 mL serological pipette tips
- Pen
- 0.22 µM PES filter
- 50 mL falcon tube rack
- 0.7% LB top agar
- P200 pipette
- P200 pipette tips
- Commensal Category 1 *Escherichia coli* K-12 strain (Reference no. LZB 035)
- LBA plate
- P10 pipette
- P10 pipette tips
- Incubator
- Fridge

Microcentrifuge:

- 2 mL tubes
- 5 mL syringe
- 2 mL tube rack

Tabletop centrifuge:

- 20 mL syringe

This week you will see whether your sample contains a phage which can kill *E. coli* K-12. To do this, we use a technique known as a spot test. Before you complete the spot test, you will need to centrifuge and filter your enriched phage sample that you created last week to remove the bacteria.

If you are using a tabletop centrifuge skip to step B. If you are using a microcentrifuge follow step A.

Step A: Centrifuge and filter (microcentrifuge)

24. Using the 10 mL serological pipette, pipette 2 mL of your enriched phage sample into a 2 mL tube.
25. Label your 2 mL tube with your initials, the date and unique sample ID. (For guidance on how to correctly label a sample, see 'General Lab Skills').
26. Place your 2 mL tube in the centrifuge.

    IMPORTANT: Make sure the centrifuge is balanced. (For guidance on how to balance a centrifuge, see 'General Lab Skills').

4. Centrifuge your enriched sample at 3000-5000x g for 15 minutes at 4°C.
5. Open the packaging containing the 0.22 µM PES filter, but leave the filter inside the packaging.
6. Place the 5 mL syringe in the PES filter (set the plunger back inside the syringe packaging to maintain aseptic conditions).

7. Place the filter and syringe over an open, clean 50 mL falcon tube.
8. Pour the supernatant from your 2 mL tube into the syringe.
   IMPORTANT: Only pour the supernatant into the syringe. Do not pour the pellet at the bottom of the 2 mL tube. (For guidance on how to correctly pour the supernatant, see 'General Lab Skills').
9. Deplunge the syringe, passing the supernatant through the filter and into the falcon tube.
10. Seal the falcon tube.
11. Write your initials, the date and unique sample ID on the falcon tube containing your filtered phage sample and store it in a falcon tube.

Scan to watch a video tutorial on Step A

Step B: Centrifuge and filter (tabletop centrifuge)

1. Place your enriched phage sample into the centrifuge.
   IMPORTANT: Make sure the centrifuge is balanced. (For guidance on how to balance a centrifuge, see 'General Lab Skills').
2. Centrifuge your enriched sample at 3000-5000x g for 15 minutes at 4°C.
3. Open the packaging containing the 0.22 µM PES filter, but leave the filter inside the packaging.
4. Place the 20 mL syringe in the PES filter (set the plunger back inside the syringe packaging to maintain aseptic conditions).
5. Place the filter and syringe over an open, clean 50 mL falcon tube.
6. Pour the supernatant from the falcon tube into the syringe.
   IMPORTANT: Only pour the supernatant into the syringe. Do not pour the pellet at the bottom of the falcon tube. (For guidance on how to correctly pour the supernatant, see 'General Lab Skills').
7. Deplunge the syringe, passing the supernatant through the filter and into the falcon tube.
8. Seal the falcon tube.
9. Write your initials, the date and unique sample ID on the falcon tube containing your filtered phage sample and store it in the falcon tube rack.

Scan to watch a video tutorial on Step B

Step C: Plate preparation

2. Pipette 5 mL of warm 0.7% LB top agar into a 50 mL falcon tube using the 10 mL serological pipette.
3. Add 100 µL of the *E. coli* K-12 culture into the falcon tube using the P200 pipette.
   IMPORTANT: Make sure that the top agar is not too hot because it will kill the bacteria.
3. Rock the falcon tube gently to mix the top agar and bacteria.
   IMPORTANT: Do not shake the falcon tube, because it will create air bubbles in the top agar which will hinder your bacterial lawn.
4. Pour the LB top agar and *E. coli* K-12 mixture onto the LBA plate to form the bacterial lawn. Make sure the top agar has covered the entire plate.
5. Cover the agar plate and let it dry for 10 minutes.
6. Once dry, write your initials, the date and unique sample ID on the bottom of the plate.

7. Using the pen, on the bottom of the plate place a dot in the centre of dish. This will mark where you will spot your phage sample in the next step.

The top agar should be 50°C so that it does not harm or kill the bacteria when you mix them. Make sure to do steps 3-6 quickly, before the top agar cools and solidifies after taking it out of the water bath.

Scan to watch a video tutorial on Step C

Step D: Spot test

1. Add 10 µL of your filtered phage sample using the P10 pipette onto the bacterial lawn.
   TIP: Place your elbows on the benchtop to help stabilise yourself when pipetting onto the plate.
2. Place the lid on the agar plate and let it dry for 10-20 minutes.
3. Incubate the plate at 25°C overnight.
4. Save your remaining filtered phage sample in the falcon tube for next week's practical. Store in a 4°C fridge.

Scan to watch a video tutorial on Step D

If your sample contains phages, you will see plaques (clear zones) on the top agar. This demonstrates where the phage has lysed (killed) the *E. coli* K-12.

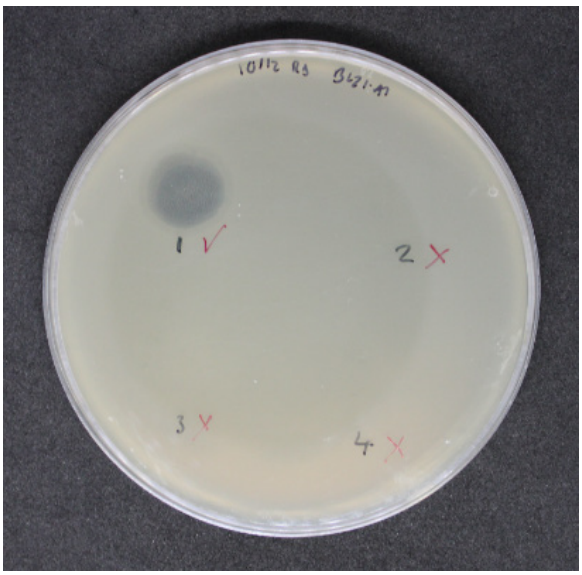


Figure 8: An example of a phage spot assay demonstrating that sample '1' contains phages which lyse *E. coli* by the production of a plaque (clear zone) on the agar plate.

NOTES

THE PHAGE COLLECTION PROJECT

WEEK 3: SERIAL DILUTION

Equipment:

- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- P200 pipette
- P200 pipette tips
- LB broth
- 96-well plate
- 50 mL falcon tube rack
- λ phage stock (if using)
- Pen
- Fridge

This week you will see whether your sample contains any phages which lyse *E. coli* K-12. If your sample is positive for a phage, you will then prepare a serial dilution of your phage sample for testing next week. A serial dilution is a stepwise series of dilutions, where the dilution factor remains the same for each step. We use a serial dilution in phage isolation to help us isolate individual phage plaques.

Step A: Plaque analysis

13. Look at the agar plate you prepared last week. Can you see clear or turbid lysis zones which indicate bacterial killing by phage?
14. If your agar plate shows phage plaques, continue to step B.
15. If your agar plate does not show any phage plaques, let your teacher or laboratory technician know. They will provide you with an agar plate they have prepared which contains Lambda (λ) phage. λ phage is a phage which infects and kills *E. coli*. You will use this phage to continue the Phage Isolation Course.

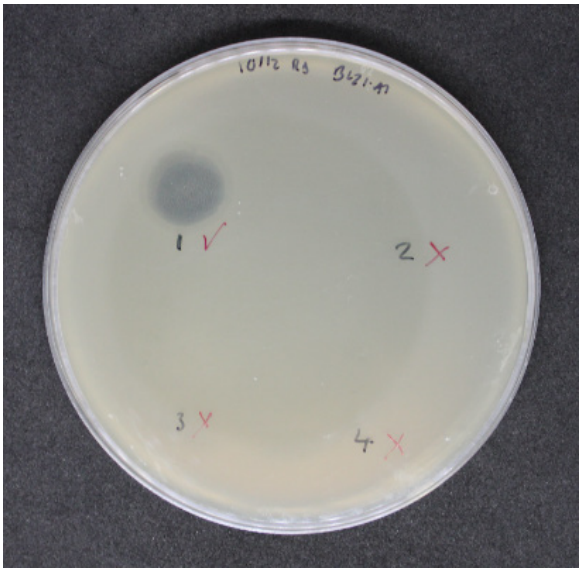


Figure 9: An example of a phage spot assay demonstrating that sample '1' contains phages which lyse *E. coli* by the production of a plaque (clear zone) on the agar plate.

After observing the phage plaques, you will create serial dilutions, using the filtered phage sample that you created last week, to see individual phage plaques. If you are using λ phage, your teacher or laboratory technician will provide you with a sample of this phage.

Step B: Serial dilution

1. Using the P200 pipette, transfer 180 µL of LB broth into the first 8 wells in the 96-well plate. (For guidance on how to pipette, see 'General Lab Skills').
2. Add 20 µL of the filtered phage sample you prepared last week into the first well in the 96-well plate using the P200 pipette.
   IMPORTANT: Make sure to change the pipette tip between step 1 and 2.
3. Mix the phage sample and LB broth by pipetting up and down.
4. Remove and change your pipette tip.
5. Pipette 20 µL from the first well into the second well in the 96-well plate and mix by pipetting up and down.
6. Repeat this process for the rest of the wells until you reach the 8th final well. Make sure to take from the well you have just diluted from each time you repeat the process (e.g. well 2 to well 3, well 3 to well 4).
   IMPORTANT: Change pipette tips between each dilution to avoid cross contamination between the wells.
7. Seal the 96-well plate and write your initials, the date and unique sample ID (or λ phage if using) on the lid.
8. Write the phage dilutions on the wells in standard form, with 10^{-1} being the least dilute, 10^{-8} being the most dilute.
9. Store the plate with the dilutions of your phage sample in a 4°C fridge.
10. Store the remaining filtered phage sample not used for the serial dilution in a 4°C fridge.

Scan to watch a video tutorial on Step B

NOTES

THE PHAGE COLLECTION PROJECT

WEEK 4: PHAGE SPOTTING

Equipment:

- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- LBA plate
- Pen
- Ruler
- 10 mL serological pipette
- 10 mL serological pipette tips
- 0.7% top agar
- 50 mL falcon tube
- 50 mL falcon tube rack
- P200 pipette
- P200 pipette tips
- Commensal Category 1 *Escherichia coli* K-12 strain (Reference no. LZB 035)
- P10 pipette
- P10 pipette tips
- λ phage stock (if using)
- Incubator

Today you will be using the serial dilutions of your phage sample that you prepared last week and testing them against *E. coli* K-12, to identify the dilution to grow and observe individual phage plaques on an agar plate.

To do this, first you need to prepare your bacterial lawn.

Step A: Plate preparation

20. Prepare your LBA plate by drawing lines to create 8 equal segments on the bottom of the plate using a pen and ruler.
21. Label the segments 1 to 8.
22. Pipette 5 mL of warm 0.7% LB top agar into a 50 mL falcon tube using the 10 mL serological pipette.
23. Add 100 µL of the *E. coli* K-12 culture into the falcon tube using the P200 pipette.
    IMPORTANT: Make sure that the top agar is not too hot because it will kill the bacteria.
5. Rock the falcon tube gently to mix the top agar and bacteria.
   IMPORTANT: Do not shake the falcon tube, because it will create air bubbles in the top agar which will hinder your bacterial lawn.
6. Pour the LB top agar and *E. coli* K-12 mixture onto the labelled LBA plate to form the bacterial lawn. Make sure the top agar has covered the entire plate.
7. Cover the agar plate and let it dry for 10 minutes.
8. Once dry, write your initials, the date and unique sample ID (or λ phage if using) on the bottom of the plate.

The top agar should be 50°C so that it does not harm or kill the bacteria when you mix them. Make sure to do steps 3-6 quickly, before the top agar cools and solidifies after taking it out of the water bath.

Scan to watch a video tutorial on Step A

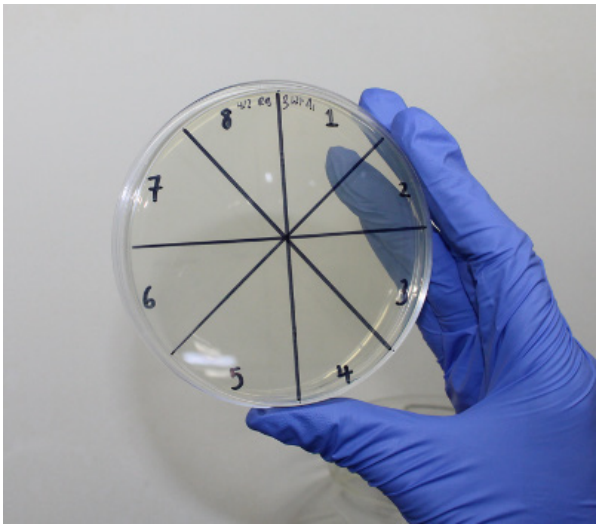


Figure 10: A LBA plate divided into 8 equal segments

Step B: Phage spotting

1. Spot 5 µL of your first phage dilution in your 96-well plate onto the bacterial lawn in zone 1 marked on the agar plate.
   TIP: Place your elbows on the bench top to help stabilise yourself when pipetting onto the plate. (For guidance on how to pipette, see 'General Lab Skills').
2. Repeat this for the rest of your phage dilutions, placing the second serial dilution into zone 2, third dilution into zone 3 etc.
   IMPORTANT: Make sure to change pipette tips when spotting each dilution onto the plate to avoid cross contamination between the zones.
3. Once you have spotted all eight serial dilutions, place the lid on the agar plate and let it dry for 10-20 minutes.
4. Incubate the plate at 25℃ overnight.

Scan to watch a video tutorial on Step B

NOTES

THE PHAGE COLLECTION PROJECT

WEEKS 5-7: PHAGE PURIFICATION

Equipment:
- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- 10 mL serological pipette
- 10 mL serological pipette tips
- 0.7% top agar
- 50 mL falcon tube
- 50 mL falcon tube rack
- P200 pipette
- P200 pipette tips
- Commensal Category 1 *Escherichia coli* K-12 strain (Reference no. LZB 035)
- LBA plate
- Pen
- Sterile toothpicks
- Sterile tweezers
- Sterile paper strips
- Incubator

Inspect the agar plate that your prepared last week. There is a chance that your sample contained multiple phages. These phages can produce different phage plaques. They may be different sizes, some may be more transparent than others, or even have a halo morphology.

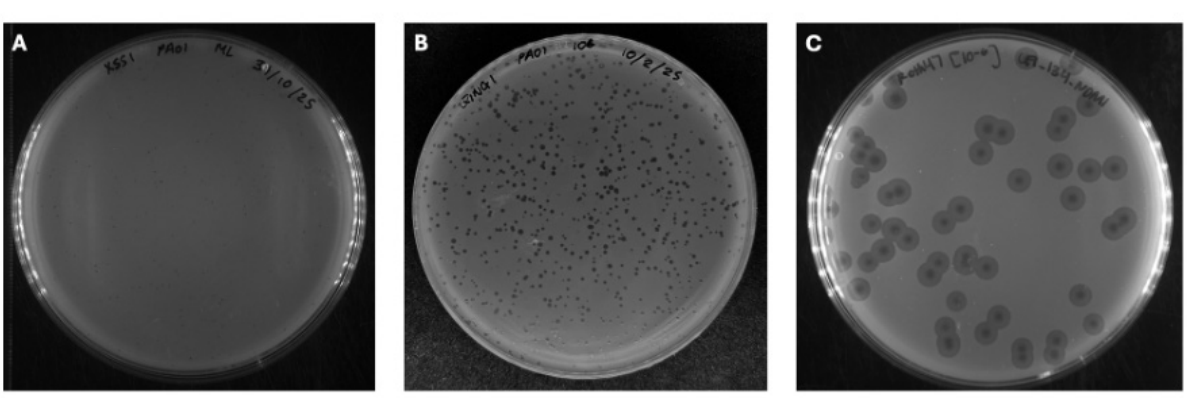


Figure 11: Agar plates with different phage plaque morphologies. Plates A and B are *Pseudomonas aeruginosa* phages. Plate C is a *Klebsiella pneumoniae* phage, which has produced a halo plaque morphology.

Over the next 3 weeks you will be purifying the phages you have isolated. Look at your agar plate. Which phage dilution produced individual plaques? Once identified, use this dilution to purify your phages.



Figure 12: An example agar plate with 8 phage dilutions. At which dilution can you see individual phage plaques on this plate?

Step A: Plate preparation

1. Pipette 5 mL of warm 0.7% LB top agar into a 50 mL falcon tube using the 10 mL serological pipette.
2. Add 100 µL of the *E. coli* K-12 culture into the falcon tube using the P200 pipette.
   IMPORTANT: Make sure that the top agar is not too hot because it will kill the bacteria.
3. Rock the falcon tube gently to mix the top agar and bacteria.
   IMPORTANT: Do not shake the falcon tube, because it will create air bubbles in the top agar which will hinder your bacterial lawn.
4. Pour the LB top agar and *E. coli* K-12 mixture onto the labelled LBA plate to form the bacterial lawn. Make sure the top agar has covered the entire plate.
5. Cover the agar plate and let it dry for 10 minutes.
6. Once dry, write your initials, the date and unique sample ID (or λ phage if using) on the bottom of the plate.

Scan to watch a video tutorial on Step A

Step B: Phage purification

3. Prick one phage plaque from the plate you created last week using a sterile toothpick.
   IMPORTANT: Select a phage plaque from the dilution where you can see individual plaques (refer to the example agar plate above).
2. Using the same toothpick, prick the top agar you prepared in Step A 8 times in a row across the plate.
3. Using sterile tweezers, slide a strip of sterile paper over the row of punctures to spread the phages across the agar plate in a straight line.
   IMPORTANT: Gently slide the strips of paper over the agar, to avoid ripping the top agar.
4. Slide another strip of sterile paper halfway across the previous line you have just created, in a straight line.

5. Repeat step 4 until you have covered the entire agar plate.
6. Incubate the plate at 25℃ overnight.

Repeat steps A and B during weeks 6 and 7, until you see the phage plaques become consistent.

Scan to watch a video tutorial on Step B

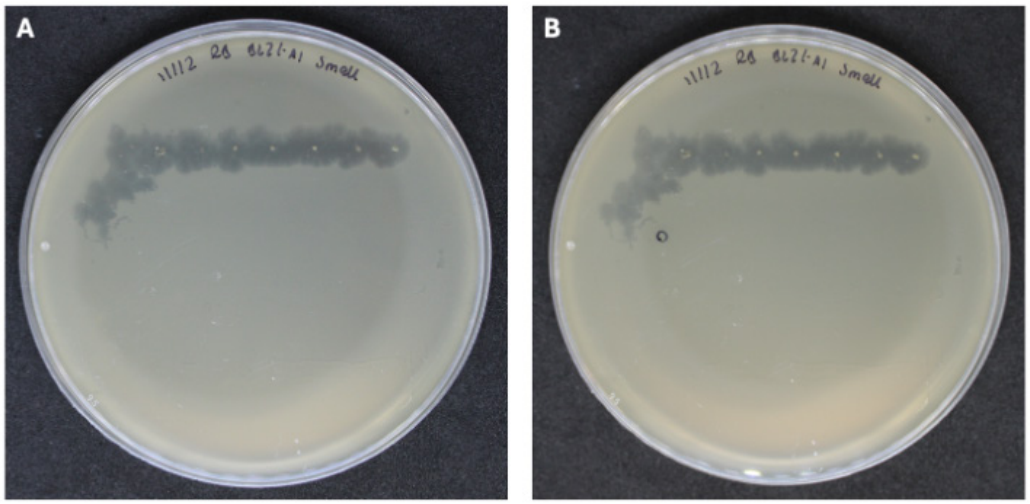


Figure 13: Agar plates showing the first round of phage purification. To prepare for the second round of purification, select an individual phage plaque to pick, as shown in plate B.

NOTES

THE PHAGE COLLECTION PROJECT

WEEK 8: PHAGE PRODUCTION

Equipment:

- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- Sterile toothpicks
- Pen
- Incubator

Now that you have purified your phage, you are going to produce it high quantities so that we can conduct further tests on it in the lab. To do this, we need to add our purified phage to the *E. coli* K-12 culture.

Step A: Phage production

8. Using a sterile toothpick, select one of the individual phage plaques from your last round of purification and add it to the 50 mL falcon tube containing the bacterial culture your teacher and laboratory technician prepared for you.
9. Seal the falcon tube and write your initials, the date and unique sample ID (or phage if using) on the tube.
10. Incubate the culture overnight at 25 C.
    OPTIONAL: Incubate with rocking if available.

Scan to watch a video tutorial on Step A

The phage will infect some of the *E. coli* cells, generating new phages that will burst from the cell and into the surrounding media. Here the new virions will encounter other *E. coli* cells to infect, generating more phages at each round of infection.

NOTES

THE PHAGE COLLECTION PROJECT

WEEK 9: PHAGE TITRATION

Equipment:

- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- Sterile tweezers
- 10 mL serological pipette
- 10 mL serological pipette tips
- Pen
- 0.22 µM PES filter
- 50 mL falcon tube
- 50 mL falcon tube rack
- P200 pipette
- P200 pipette tips
- LB broth
- 96-well plate
- Fridge

Microcentrifuge:

- 2 mL tubes
- 5 mL syringe
- 2 mL tube rack

Tabletop centrifuge:

- 20 mL syringe

This week you will be preparing a phage titration. This is a method that enables us to determine the concentration of phages in a sample. First, we need to centrifuge and remove the bacteria from the sample to create a purified phage sample.

If you are using a tabletop centrifuge skip to step B. If you are using a microcentrifuge follow step A.

Step A: Centrifuge and filter (microcentrifuge)

21. Remove the toothpick from the enriched phage sample you created last week using sterile tweezers and dispose of responsibly.
22. Using the 10 mL serological pipette, pipette 2 mL of you enriched phage sample into a 2 mL tube.
23. Label your 2 mL tube with your initials, the date and unique sample ID (or λ phage if using). (For guidance on how to correctly label a sample, see 'General Lab Skills').
24. Place your 2 mL tube in the centrifuge.
    IMPORTANT: Make sure the centrifuge is balanced. (For guidance on how to balance a centrifuge, see 'General Lab Skills').
5. Centrifuge your enriched sample at 3000-5000x g for 15 minutes at 4°C.
6. Open the packaging containing the 0.22 µM PES filter, but leave the filter inside the packaging.
7. Place the 5 mL syringe in the PES filter (set the plunger back inside the syringe packaging to maintain aseptic conditions).
8. Place the filter and syringe over an open, clean 50 mL falcon tube.

9. Pour the supernatant from your 2 mL tube into the syringe.
   IMPORTANT: Only pour the supernatant into the syringe. Do not pour the pellet at the bottom of the 2 mL tube. (For guidance on how to correctly pour the supernatant, see 'General Lab Skills').
10. Deplunge the syringe, passing the supernatant through the filter and into the falcon tube.
11. Seal the falcon tube.
12. Write your initials, the date and unique sample ID (or λ phage if using) on the falcon tube containing your filtered phage sample and store it in the falcon tube rack.

Scan to watch a video tutorial on Step A

Step B: Centrifuge and filter (tabletop centrifuge)

1. Remove the toothpick from the enriched phage sample you created last week using sterile tweezers and dispose of responsibly.
2. Place your enriched phage sample into the centrifuge.
   IMPORTANT: Make sure the centrifuge is balanced. (For guidance on how to balance a centrifuge, see 'General Lab Skills').
3. Centrifuge your enriched sample at 3000-5000x g for 15 minutes at 4°C.
4. Open the packaging containing the 0.22 µM PES filter, but leave the filter inside the packaging.
5. Place the 20 mL syringe in the PES filter (set the plunger back inside the syringe packaging to maintain aseptic conditions).
6. Place the filter and syringe over an open, clean 50 mL falcon tube.
7. Pour the supernatant from your falcon tube into the syringe.
   IMPORTANT: Only pour the supernatant into the syringe. Do not pour the pellet at the bottom of the falcon tube. (For guidance on how to correctly pour the supernatant, see 'General Lab Skills').
8. Deplunge the syringe, passing the supernatant through the filter and into the falcon tube.
9. Seal the falcon tube.
10. Write your initials, the date and unique sample ID (or λ phage if using) on the falcon tube containing your filtered phage sample and store it in the falcon tube rack.

Scan to watch a video tutorial on Step B

Step C: Serial dilution

3. Using the P200 pipette, transfer 180 µL of LB broth to the first 8 wells in the 96-well plate.
4. Add 20 µL of the filtered phage sample you have just prepared into the first well in the 96-well plate.
   IMPORTANT: Make sure to change the pipette tips between step 1 and 2.
3. Mix the phage sample and LB broth by pipetting up and down.
4. Remove and change your pipette tip.
5. Pipette 20 µL from the first well into the second well in the 96-well plate and mix by pipetting up and down.
6. Repeat this process for the rest of the wells until you reach the 8th final well. Make sure to take from the well you have just diluted from each time you repeat the process (e.g. well 2 to well 3, well 3 to well 4).

IMPORTANT: Make sure to change pipette tips between each dilution to avoid cross contamination between the wells.

7. Seal the 96-well plate and write your initials, the date and unique sample ID (or λ phage if using) on the lid.
8. Write the phage dilutions on the wells in standard form, with 10^{-1} being the least dilute, 10^{-8} being the most dilute.
9. Store the 96-well plate with the dilutions of your phage sample in a 4°C fridge.
10. Store the remaining filtered phage sample not used for the serial dilution in a 4°C fridge.

Scan to watch a video tutorial on Step C

NOTES

THE PHAGE COLLECTION PROJECT

WEEK 10: PHAGE TITRATION

Equipment:

- 70% ethanol
- Bunsen burner
- Heatproof mat
- Matches
- LBA plate
- Pen
- Ruler
- 10 mL serological pipette
- 10 mL serological pipette tips
- 0.7% LB top agar
- 50 mL falcon tube
- 50 mL falcon tube rack
- P200 pipette
- P200 pipette tips
- Commensal Category 1 *Escherichia coli* K-12 strain (Reference no. LZB 035)
- P10 pipette
- P10 pipette tips
- Incubator

Using the serial dilutions you prepared last week, you are going to determine the concentration of the phage you have isolated and purified. Like in week 4, we do this by first preparing the agar plate and then spotting the different dilutions of our phage.

Step A: Plate preparation

19. Prepare your LBA plate by drawing lines to create 8 equal segments on the bottom of the plate using a pen and ruler.
20. Label the segments 1 to 8.
21. Pipette 5 mL of warm 0.7% LB top agar into a 50 mL falcon tube using the 10 mL serological pipette.
22. Add 100 µL of the *E. coli* K-12 culture into the falcon tube using the P200 pipette.
    IMPORTANT: Make sure that the top agar is not too hot because it will kill the bacteria.
5. Rock the falcon tube gently to mix the top agar and bacteria.
   IMPORTANT: Do not shake the falcon tube, because it will create air bubbles in the top agar which will hinder your bacterial lawn.
6. Pour the LB top agar and *E. coli* K-12 mixture onto the LBA plate to form the bacterial lawn. Make sure the top agar has covered the entire plate.
7. Cover the agar plate and let it dry for 10 minutes.
8. Once dry, write your initials, the date and unique sample ID (or λ phage if using) on the bottom of the plate.

The top agar should be 50℃ so that it does not harm or kill the bacteria when you mix them. Make sure to do steps 3-6 quickly, before the top agar cools and solidifies after taking it out of the water bath.

Scan to watch a video tutorial on Step A

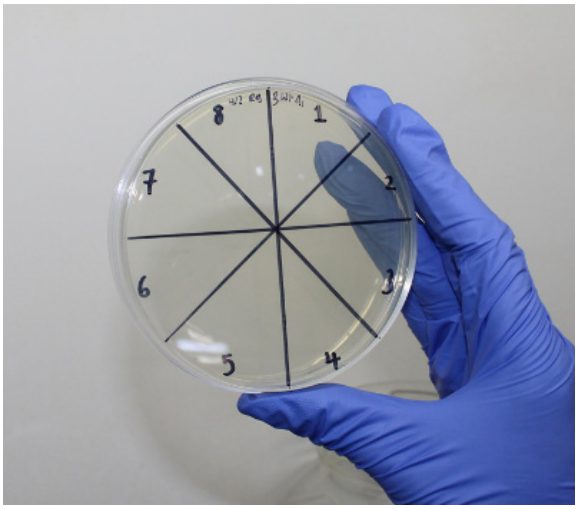


Figure 14: An agar plate divided into 8 equal parts.

Step B: Phage spotting

1. Spot 5 µL of your first phage dilution in your 96-well plate onto the bacterial lawn in zone 1 marked on the agar plate using a P10 pipette.
2. Repeat this for the rest of your phage dilutions, placing the second serial dilution into zone 2, third dilution into zone 3 etc.
   IMPORTANT: Make sure to change pipette tips when spotting each dilution onto the plate to avoid cross contamination between the zones.
3. Once you have spotted all eight serial dilutions, place the lid on the agar plate and let it dry for 10-20 minutes.
4. Incubate the plate at 25℃ overnight.

Scan to watch a video tutorial on Step B

NOTES

THE PHAGE COLLECTION PROJECT

WEEK 11: PHAGE TITRATION

Equipment:
- 70% ethanol
- Scientific calculator

In the final week of the course, you will be using the agar plate you made last week to determine how well your purified phage has replicated and the concentration of the phage stock you have produced.

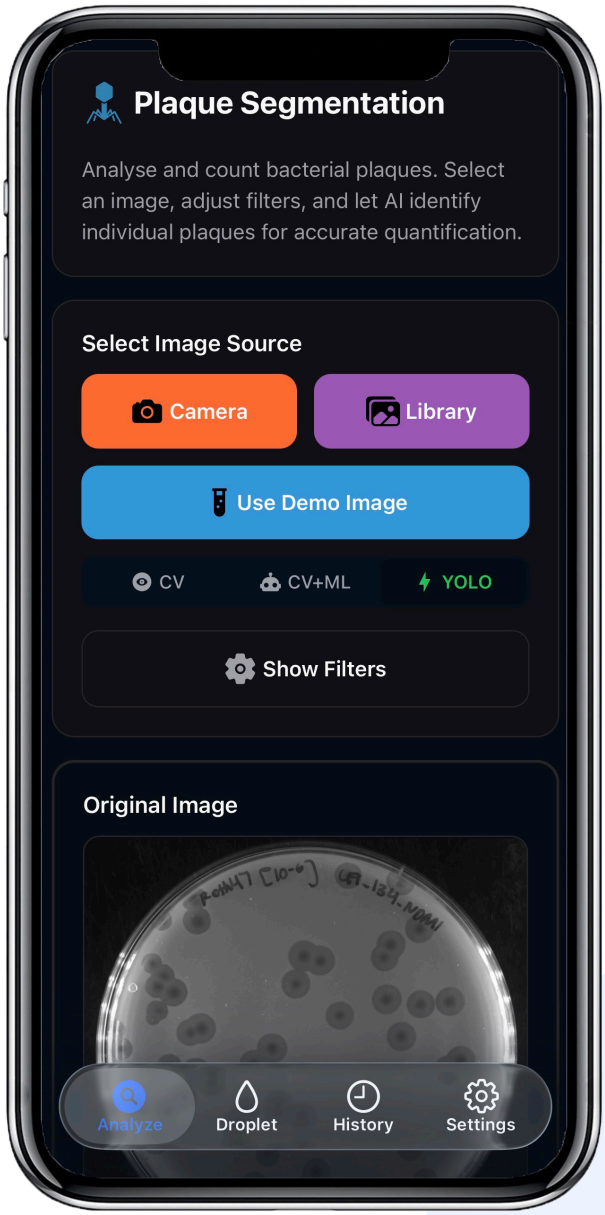
Step A: Plaque analysis

3. Look at the agar plate you prepared last week. Look for the dilution where you can count between 3-30 individual phage plaques.

Check out and use our Plaque Segmentation app to help you count your phage plaques!

Scan the QR code to download the app on an iPhone

Scan the QR code to download the app on an Android device



THE PHAGE COLLECTION PROJECT

Step B: Concentration calculation

1. Using the formula below, calculate the concentration of your phage stock.

$$Phage\ concentration\ (pfu/mL) = \frac{Number\ of\ phage\ plaques\ \times\ 10^{dilution}}{volume\ of\ sample\ plated\ (mL)}$$

EXAMPLE:

In the plate below, you can count 5 individual phage plaques in dilution 10^{-6}. As you remember from last week, you plated 5 µL (0.005 mL) of your phage sample. Therefore, the formula would become:



Figure 15: An example agar plate with 8 phage dilutions. Use this example plate to help you calculate your own phage concentration.

$$Phage\ concentration = \frac{5\ \times\ 10^{6}}{0.005} = 1\ \times\ 10^{9}\ pfu/mL$$

NOTES

THE PHAGE COLLECTION PROJECT

CONGRATULATIONS

Congratulations on isolating a phage and completing the Phage Isolation Course!

If you isolated a phage from your 50mL water sample that you collected at the beginning of the course, it is now time to name your phage! On the falcon tube containing your purified phage sample that you created in week 9, write the name that you would like to give to your phage. Please find some guidance for naming your phage below.

Phage naming guidance:
- Between 4 -14 characters in length
- Letters and numbers only (no special characters)
- No spaces
- Do not use an individual's full name e.g. TomBaker (Tom, or Baker alone are acceptable)
- Pronounceable name e.g. ROTH78

DISCLAIMER: If The Phage Collection Project receives identical names, we will introduce a unique reference number to that name.

To submit your phage name to The Phage Collection Project by scanning the QR code below:

Through your involvement with the project, you have participated in active scientific research aimed at combatting antibiotic-resistant infections. The phages you have isolated will help us to better understand the therapeutic application of these viruses and may be used to help treat a patient struggling with a chronic bacterial infection.

Our team looks forward to conducting further experiments on your phage and sharing the findings with your school. Make sure to also check out our project portal on our website, an open-resource virtual biobank, to find your phage.

Thank you for your contribution and for being an integral part of our research community.

The Phage Collection Project Team

phage-collection.org

phage.collection

The Phage Collection Project

phagecollection.bsky.social

THE PHAGE COLLECTION PROJECT

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